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Induction of Metamorphosis of Larvae of the Green Sea Urchin, *Strongylocentrotus droebachiensis*, by Coralline Red Algae

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Abstract. The coralline red algae, *Lithothamnion glaciale, Phymatolithon laevigatum, P. rugulosum,* and *Corallina officinalis,* induced >85% of laboratory-reared larvae of *Strongylocentrotus droebachiensis* to metamorphose. Larvae must contact live *L. glaciale* or its spores for metamorphosis to occur; the inducer is not sensed in the water column. However, aqueous extracts of *L. glaciale* can induce metamorphosis, suggesting that the inducing factor is chemical. Neither ashed nor boiled *L. glaciale* induces metamorphosis, indicating that the factor is heat-labile and that thigmotaxis, per se, is not important in the response. The amino-acid, γ-aminobutyric acid (GABA), which induces settlement of other marine invertebrate larvae, also induces significant rates of metamorphosis of *S. droebachiensis* at concentrations $\geq 10^{-4}$ *M*. A reduction (with antibiotics) in the number of live bacteria on the surface of *L. glaciale* does not affect the rate of metamorphosis of larvae.

Introduction

The larvae of a variety of benthic marine invertebrates are known to settle and metamorphose in response to coralline red algae, including: corals, *Agaricia agaricites danai, A. agaricites humilis,* and *A. tenuifolia* (Morse *et al.*, 1988); chitons, *Tonicella lineata* (Barnes and Gonor, 1973), *Mopalia muscosa* (Morse *et al.*, 1979a), and *Katharina tunicata* (Rumrill and Cameron, 1983); limpets, *Acmaea testudinalis* (Steneck, 1982); trochid gastropods, *Trochus niloticus* (Heslinga, 1981); abalone, *Haliotis* spp. (Shepherd, 1973; Morse *et al.*, 1979a, 1980a; Morse and Morse, 1984; Shepherd and Turner, 1985); tubeworms, *Spirorbis corallinae* (de Silva, 1962) and *S. rupestris* (Gee, 1965); sea urchins, *Strongylocentrotus purpuratus* (Rowley, 1989); and seastars, *Acanthaster planci* (Henderson and Lucas, 1971; Yamaguchi, 1973; Lucas and Jones, 1976) and *Stichaster australis* (Barker, 1977).

The relationship between some grazers and coralline algae may be mutually beneficial, and the species may have co-evolved. For example, by preferentially settling and metamorphosing on crustose coralline algae, the abalone *Haliotis rufescens* gains obligate chemical cues for the induction of metamorphosis and further development, micro-refuges from predation, adequate food to support early growth (*e.g.,* mucous exudates of the coralline alga, diatoms, bacteria, and other epiphytes), and camouflage (the red pigment of the coralline alga is incorporated into the shell of the developing abalone). In turn, the coralline alga is cleaned of epiphytic algae (which reduce photosynthesis and can potentially kill the coralline) by the abalone's grazing activity (Morse *et al.*, 1980a; Morse and Morse, 1984). A similar, mutualistic relationship has been shown with the limpet, *Acmaea testudinalis,* and the coralline alga, *Clathromorphum circumscriptum* (Steneck, 1982).

In the shallow rocky subtidal zone of north temperate oceans, strongylocentrotid sea urchins are generally associated with coralline algal-dominated communities, described by various workers as "barren grounds" (Pearse *et al.*, 1970; Lawrence, 1975), "Isoyake areas" (Hagen, 1983), or "coralline flats" (Ayling, 1981). In many cases, the destructive grazing of kelps and other fleshy macroalgae by expanding populations of sea urchins has led to the establishment of these coralline communities, which are maintained by continued intensive grazing (see reviews by Lawrence, 1975; Lawrence and Sammarco, 1982; and Chapman, 1986). This has been well documented for *Strongylocentrotus droebachiensis* in eastern Canada

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(Mann and Breen, 1972; Breen and Mann, 1976a, b; Lang and Mann, 1976; Mann, 1977; Breen, 1980; Chapman, 1981; Wharton and Mann, 1981), where the common shallow-water species of coralline algae are *Clathromorphum circumscriptum, Corallina officinalis, Lithothamnion glaciale, Phymatolithon laevigatum,* and *P. rugulosum.* Recruitment of *S. droebachiensis* (Lang and Mann, 1976; Wharton and Mann, 1981; Miller, 1985; Scheibling, 1986) and other strongylocentrotid species (Pearse *et al.*, 1970; Tegner and Dayton, 1981) is lower in kelp beds than in coralline barren grounds, and selective settlement of sea urchin larvae on coralline substrata may account, at least in part, for these differences (Raymond and Scheibling, 1987).

In this study, we show that larvae of *S. droebachiensis* are induced to settle and metamorphose in the presence of coralline algae. In a series of laboratory experiments with *L. glaciale,* we investigate the potential mechanism of settlement induction. We discuss the implications of this result to settlement patterns in the field.

Materials and Methods

Larval rearing

Adults of *Strongylocentrotus droebachiensis* were collected at 5–10 m depth at Sandy Cove (Digby County), Nova Scotia, Canada (44° 29′ N, 66° 05′ W). They were maintained in the laboratory in running seawater and fed kelp (*Laminaria digitata* and *L. longicruris*) at regular intervals.

Gametes from adults of *S. droebachiensis* (50–85 mm test diameter) were obtained by peristomial injection of 2.5–4.0 ml of 0.53 *M* KCl. Females shed their eggs into glass bowls of chilled 0.45 μm Millipore®-filtered seawater (hereafter referred to as filtered seawater); males shed sperm into dry, chilled bowls. After ~20 min of spawning, the eggs were rinsed three to four times with filtered seawater. Several drops of sperm (checked under a microscope for motility) from one male were mixed with the eggs from one female for ~10 min. The eggs were then rinsed another three to four times with filtered seawater. Mean (±SD) percentage of fertilized eggs, as judged by the presence of a fertilization membrane, was 99.4 ± 0.6% (n = 15).

Early-stage embryos were reared in standing cultures in small glass bowls for ~72–120 h post-fertilization. When blastulae were seen swimming at the surface of the water, they were transferred to 4-l glass jars containing ~3 l of filtered seawater which was stirred constantly by T-paddles attached to 10-rpm motors. Larval densities, after the first week in stirred cultures, were maintained at ≤2 individuals ml^{-1}. All culturing was carried out in filtered seawater at 10.8 ± 1.4°C (mean ± SD, n = 535), approximating ambient seawater temperatures off Nova Scotia in June and July when larvae of *S. droebachiensis* are settling (Raymond and Scheibling, 1987). Fluorescent lighting provided a light intensity (at culture jar level) of 99.6 ± 11.6 $\mu E\ m^{-2}s^{-1}$ (mean ± SD, n = 3) on a 12 L:12 D photoperiod. Every second day (occasionally every third day) 50–75% of the culture water was removed by reverse filtration and replaced with fresh filtered seawater and microalgal food. The larvae were fed *Dunaliella tertiolecta* (a unicellular green alga) at a concentration of 1×10^4 cells ml^{-1} of culture water. Algae were cultured at 23°C under constant fluorescent illumination in f/2 nutrient medium (Guillard and Ryther, 1962). Only larvae that were deemed competent were used in experiments. Competence was indicated by the presence of large juvenile rudiments and a high rate of metamorphosis (>60%) in trial assays with coralline algae. The time from fertilization to competency ranged from 33 to 51 days.

Experimental protocols

For any experiment, only larvae from the same batch were used. If more than one culture jar of larvae was required for an experiment, larvae from different jars were thoroughly mixed before allocation to treatments. Experiments were run in 250-ml glass jars with ~150 ml of filtered seawater and a test substratum or ~150 ml of a test solution. Five replicate jars (each with 25 larvae) were used per treatment (except where noted). Larvae were transferred into experimental jars with a syringe. Experiments were run for ~24 h (range: 24–28 h) in the same environmental chamber and at the same temperature and photoperiod as larval cultures. The light intensity at experimental jar level (shaded during the light period) was 2.8 ± 0.1 $\mu E\ m^{-2}s^{-1}$ (mean ± SD, n = 3).

After 24 h, larvae and recently metamorphosed juveniles were located in jars using a dissecting microscope and classified as: (1) free-swimming (larvae only), (2) on test alga (when an algal substratum was present), or (3) on bottom or sides of experimental jar. To facilitate the location of recently metamorphosed individuals (221–392 μm test diameter) on coralline algal substrata, the following technique was used. After counting and removing any free-swimming larvae from a jar, the coralline algal substratum was removed and immersed in an isotonic solution of MgCl in water (72 g ml^{-1}) to narcotize any juveniles or larvae on the alga. These would then easily be displaced by gentle agitation or washing of the substratum. In some cases, 3–5 ml of buffered 10% formalin in seawater were added to the MgCl samples so that counting could be postponed. Because counting was time consuming (requiring 2–14 h), replicates were set up in a completely randomized block design, and blocks of treatments were counted in succession. However, only one experiment (with coralline algal extract) had a significant block

effect, indicating that the majority of larvae that metamorphosed did so during the experimental period and not during subsequent counting of individuals.

Two controls were used for each experiment: (1) filtered seawater without any test substratum, to ensure that larvae were not metamorphosing in response to handling procedures or other unknown factors, and (2) a cobble encrusted with *Lithothamnion glaciale* (occasionally *Phymatolithon laevigatum* or *P. rugulosum*), to assess the proportion of larvae capable of metamorphosing, because the rate of metamorphosis is generally maximal in response to coralline algae (see Results).

The rate of metamorphosis was expressed as the number of individuals metamorphosed divided by the total number of individuals recovered (n) (usually ≥90% of individuals were recovered). An individual was scored as metamorphosed if the larval arms had been resorbed and the globular test, tube feet, and spines of the juvenile were apparent.

To compare the rate of metamorphosis in response to morphologically different types of coralline red algae, *Corallina officinalis* (finely branched, arborescent form), *Lithothamnion glaciale* (rugose, crustose form), and *Phymatolithon laevigatum* or *P. rugulosum* (smooth, crustose forms) (the latter two species were not distinguished and hereafter are referred to collectively as *Phymatolithon*) were collected subtidally at Eagle Head (44° 04′ N, 64° 36′ W) and Mill Cove (44° 36′ N, 64° 04′ W), Nova Scotia. *Lithothamnion glaciale* and *Phymatolithon* were collected as monocultures totally encrusting cobbles. Cobble sizes were: length, 34.6–56.4 mm; width, 24.0–44.1 mm; height, 12.9–38.5 mm. Tufts of *C. officinalis*, of similar dimensions, were presented upright in experiments. Algae were immediately transported to the laboratory in coolers where they were maintained in separate 91 × 61 × 45 cm fiberglass aquaria with running seawater. All algae were carefully cleaned of epibionts and debris and thoroughly rinsed with filtered seawater prior to use in experiments. *Lithothamnion glaciale* and *Phymatolithon* also were scrubbed with a stiff plastic brush.

To examine the effect of surface contour, in the absence of living tissue, on metamorphosis of *S. droebachiensis*, *L. glaciale* was killed either by ashing at 500°C for 4 h in a muffle furnace or by vigorous boiling in deionized water for two 15-min periods. Killed *L. glaciale* was washed in running seawater prior to experimental use (ashed for 7 days, boiled for 30 min).

To test whether *L. glaciale* released a chemical into the water that could induce metamorphosis of free-swimming larvae, five cobbles encrusted with *L. glaciale* were placed in 2 l of filtered seawater in the environmental chamber for 24 h. The *Lithothamnion*-conditioned filtered seawater was then decanted and used in an experiment with filtered seawater and *L. glaciale* controls.

To test whether urchin larvae metamorphosed in response to a diffusion gradient of inducer molecules surrounding *L. glaciale*, treatments with *Lithothamnion*-encrusted cobbles conducted under static and agitated (on a shaker table at 126 rpm) conditions were compared (this was the lowest possible speed of rotation capable of totally dispersing 1 ml of concentrated methylene blue dye in 150 ml of fresh water in under 10 min in test trials).

To test whether a water-soluble extract of *L. glaciale* would induce metamorphosis of urchin larvae, fragments of the alga were chiselled off of cobbles, scrubbed with a brush, and washed with seawater. Four hundred grams of cleaned *L. glaciale* were finely ground up in 800 ml of filtered seawater (at 11–15°C) with a mortar and pestle. The supernatant was decanted and refrigerated overnight at ~4°C, then centrifuged at 27138 × *g* for 10 min at 2–3°C to remove particulates. To test whether larvae responded in a concentration-dependent manner, this supernatant was then serially diluted to 1:5, 1:10, 1:100, 1:1000, 1:10,000, and 1:100,000 with filtered seawater. These dilutions were left overnight in the environmental chamber, and the following day 150 ml of each dilution were added to experimental jar replicates along with larvae. Protein concentration of the undiluted crude extract, as measured at the onset of the experiment using a Sigma Diagnostics® micro-protein determination kit, was 305 $\mu g\ ml^{-1}$.

The amino-acid neurotransmitter, γ-aminobutyric acid (GABA) is known to induce settlement of several benthic marine invertebrates including the chitons, *Mopalia muscosa* (Morse *et al.*, 1979a) and *Katharina tunicata* (Rumrill and Cameron, 1983), and several species of abalone of the genus *Haliotis* (*cf.* Morse, 1984); GABA-mimetic molecules, present in coralline red algae, have been shown to be the inducers of metamorphosis in *H. rufescens* (Morse *et al.*, 1979a, b, 1980b; Morse and Morse, 1984; Morse, 1985). To test whether induction of metamorphosis of urchin larvae by coralline algae could also be mimicked by GABA, solutions of GABA (obtained from the Sigma Chemical Company) were prepared in filtered seawater and tested for their ability to induce metamorphosis of larvae of *S. droebachiensis* over the concentration range of 10^{-7}–10^{-1} *M*; 10 larvae per replicate were tested.

During an experiment, *Lithothamnion glaciale* occasionally released minute spores (mean diameter ± SD: 109 ± 16 μm, n = 250) that were found on the bottom of jars. To test whether these spores could induce metamorphosis of larvae, a *Lithothamnion*-encrusted cobble was placed in each of 20 experimental jars with filtered seawater for 78 h and the spores collected. The five jars with the most spores (>50) were rinsed three to four times with filtered seawater (spores stayed attached to glass) and

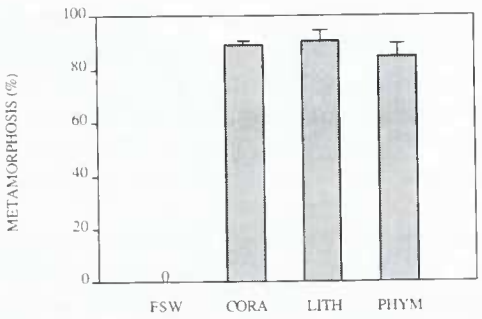

Figure 1. Mean percentage of *Strongylocentrotus droebachiensis* larvae that metamorphosed in response to filtered seawater (FSW) and the coralline red algae, *Corallina officinalis* (CORA), *Lithothamnion glaciale* (LITH), and *Phymatolithon* (PHYM). Each treatment consists of 5 replicates with 25 larvae per replicate. Error bars indicate standard error. 0 denotes that no metamorphosed individuals were found.

used as a treatment in an experiment with filtered seawater and *L. glaciale* controls.

To test whether a reduction in the number of live bacteria on the surface of *L. glaciale* would reduce the rate of metamorphosis of larvae, five *Lithothamnion*-encrusted cobbles were scrubbed with a brush, rinsed with filtered seawater, and put in 1 l of unfiltered seawater containing a mixture of penicillin and streptomycin (1000 units ml^{-1} each). After 42 h, the cobbles were removed (with sterile gloves) and rinsed with filtered seawater to remove the antibiotics and dead bacteria. Bacterial samples were collected from three antibiotic-treated cobbles and three untreated cobbles (which had been similarly scrubbed and rinsed) by swabbing a 1-cm^2 area twice (for 1 min each) with a cotton swab. The adherent material was suspended in 5 ml of artificial seawater and serially diluted in artificial seawater before being plated on marine agar plates. Bacterial colonies were counted after 5 days of development at room temperature. After swabbing, the cobbles were placed in filtered seawater (antibiotic-treated and untreated pieces in separate containers) and left overnight in the environmental chamber before use in the experiment.

All statistical tests were carried out on arcsine-transformed data. This transformation helped to normalize the data and reduce heteroscedasticity. Replicates that had 0/n (no) or n/n (all) larvae metamorphosed were replaced with values of 1/4n and 1–1/4n, respectively, to improve the transformation (Bartlett, 1937). Normality was judged by examination of cumulative probability plots, and heterogeneity of variances was assessed with Cochran's test ($\alpha = 0.01$). All statistical analyses were carried out with the SYSTAT™ (Wilkinson, 1986) statistical computer package. Untransformed values are presented in graphs.

Results

Larvae of *Strongylocentrotus droebachiensis* showed similar, high rates of metamorphosis in response to three morphologically different coralline algae: *Corallina officinalis*, *Lithothamnion glaciale*, and *Phymatolithon* (Fig. 1). Differences in mean rates among coralline treatments (range: 85–91%) were not statistically significant ($F_{2,12} = 0.45$, $P > 0.05$), indicating that morphology does not affect metamorphic rate under static laboratory conditions. No larvae metamorphosed in a concurrent filtered seawater control, indicating the requirement for an external cue.

The mean rate of metamorphosis of *S. droebachiensis* in response to live *L. glaciale* did not differ significantly among different batches of larvae from different parentage (range: 62–98%, grand mean ± SE: 86.9 ± 2.6%, n = 18) (Kruskal-Wallis test, $P > 0.05$). There also was no significant difference among these batches of larvae in their response to concurrent filtered seawater controls (range: 0–10%, grand mean ± SE: 2.3 ± 0.6%, n = 18) (Kruskal-Wallis test, $P > 0.05$).

In experiments with *L. glaciale*, killing the coralline alga markedly reduced the numbers of metamorphosing larvae (Fig. 2). The rate of metamorphosis with ashed *L. glaciale* was less than a tenth of that with live *L. glaciale*, although it was significantly greater than that in a filtered seawater control (Mann-Whitney U-test, $P < 0.05$). There was no significant difference in the rate of metamorphosis between the filtered seawater control and boiled *L. glaciale* (Mann-Whitney U-test, $P > 0.05$). Neither ashing nor boiling appeared to alter the macroscopic structure of *L. glaciale*.

Metamorphosis in *Lithothamnion*-conditioned filtered seawater was not significantly different from a filtered seawater control (Mann-Whitney U-test, $P > 0.05$), indicating that inducers are not leaking into surrounding seawater (Fig. 3). Thus, metamorphosis of urchin larvae in response to *L. glaciale* appears to require contact with the alga. The larvae are probably not responding to a diffusion gradient of inducer about live *L. glaciale*, since mild agitation

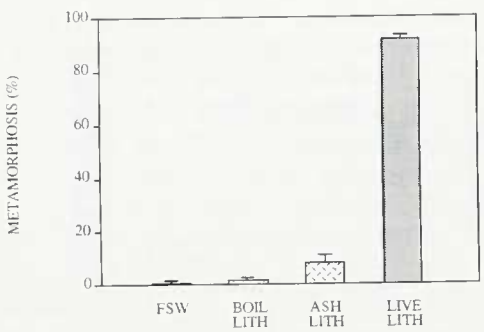

Figure 2. Mean percentage of *Strongylocentrotus droebachiensis* larvae that metamorphosed in response to filtered seawater (FSW), boiled *Lithothamnion glaciale* (BOIL LITH), ashed *L. glaciale* (ASH LITH), and live *L. glaciale* (LIVE LITH). Each treatment consists of 5 replicates with 25 larvae per replicate. Error bars indicate standard error.

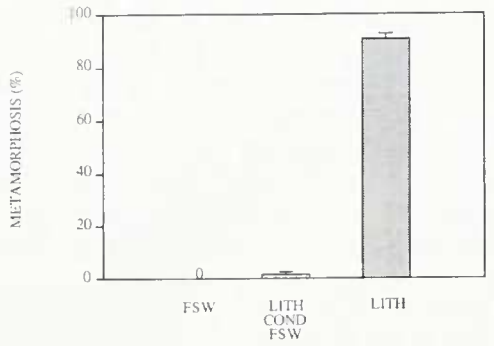

Figure 3. Mean percentage of *Strongylocentrotus droebachiensis* larvae that metamorphosed in response to filtered seawater (FSW), filtered seawater conditioned with live *Lithothamnion glaciale* (LITH COND FSW), and *L. glaciale* (LITH). Each treatment consists of 5 replicates with 25 larvae per replicate. Error bars indicate standard error. 0 denotes that no metamorphosed individuals were found.

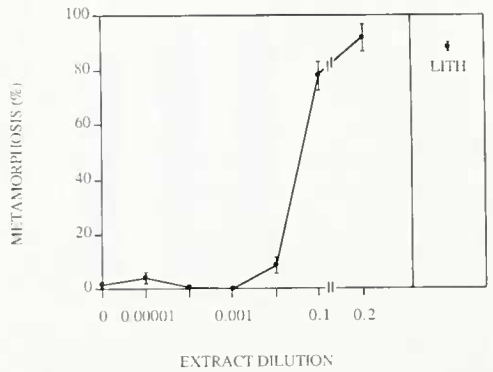

Figure 5. Mean percentage of *Strongylocentrotus droebachiensis* larvae that metamorphosed in response to serial dilutions of an extract of *Lithothamnion glaciale* and to intact *L. glaciale* (LITH). Each treatment consists of 5 replicates with 25 larvae per replicate. Error bars indicate standard error.

(which would disrupt any such gradient) did not reduce the rate of metamorphosis with *L. glaciale* (Mann-Whitney U-test, $P > 0.05$) (Fig. 4). This result provides further evidence of contact dependence.

Induction of larval metamorphosis in *S. droebachiensis* by a crude extract of *L. glaciale* in filtered seawater was concentration-dependent (Fig. 5); high rates of metamorphosis occurred at 1:5 (92%) and 1:10 (78%) dilutions, and these rates did not differ significantly from that with intact *L. glaciale* (88%) (Mann-Whitney U-test, $P > 0.05$ for both comparisons). Metamorphosis was minimal (<9%) at higher dilutions. The protein concentration of the algal extract within the range of effectiveness was between ~30 μg ml^{-1} (1:10 dilution) and 60 μg ml^{-1} (1:5 dilution).

Induction by GABA also was concentration-dependent (Fig. 6). GABA induced larval metamorphosis at concentrations $\geq 10^{-5}$ *M*; the weakest concentration of GABA that induced metamorphosis in a proportion of larvae similar to that of a coralline algal control (*Phymatolithon*) was 10^{-3} *M* (Dunnett's test, $P > 0.05$). About 20 juveniles that metamorphosed in response to GABA were placed in running seawater and observed for a period of about 2 weeks. They appeared normal and active during this time.

Larvae metamorphosed in response to spores of *L. glaciale* adhering to the glass bottom of jars. The rate of metamorphosis in a treatment with spores was significantly higher than that in a filtered seawater control (Mann-Whitney U-test, $P < 0.05$), but significantly lower than with live *L. glaciale* (Mann-Whitney U-test, $P < 0.01$) (Fig. 7). This latter result may be explained by the surface area covered by spores which was only a small fraction of that covered by the alga (spores: <1 mm^2; *L. glaciale:* >900 mm^2). Settlement and metamorphosis in response to spores may have accounted for some of the

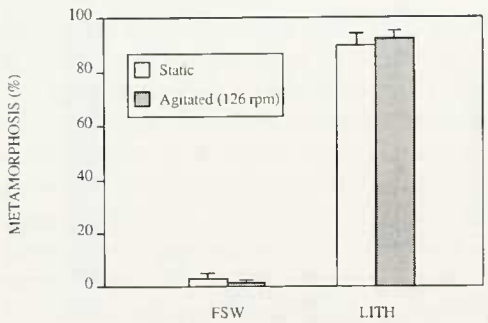

Figure 4. Mean percentage of *Strongylocentrotus droebachiensis* larvae that metamorphosed in response to filtered seawater (FSW) and *Lithothamnion glaciale* (LITH) under static (light bars) and agitated (dark bars) conditions. Each treatment consists of 5 replicates with 25 larvae per replicate. Error bars indicate standard error.

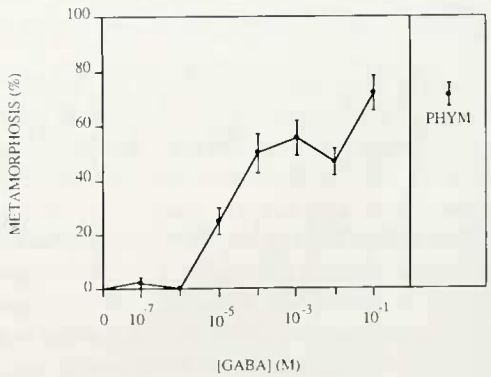

Figure 6. Mean percentage of *Strongylocentrotus droebachiensis* larvae that metamorphosed in response to various concentrations of γ-aminobutyric acid (GABA) and a *Phymatolithon* control (PHYM). Each treatment consists of 5 replicates with 10 larvae per replicate. Error bars indicate standard error.

recently metamorphosed individuals found on the bottom and sides of experimental jars after treatment with live *L. glaciale*. In treatments with live *L. glaciale* (pooled from 21 experiments), 24.5 ± 3.2% (mean ± SE) of all individuals were juveniles located on the bottom or sides of jars, whereas 60.9 ± 3.6% (mean ± SE) were juveniles on the alga.

Treating *L. glaciale* with antibiotics did not affect the rate of metamorphosis of *S. droebachiensis* (Fig. 8), although live bacterial numbers were significantly reduced with antibiotics (mean ± SD, treated: $5.60 \times 10^2 \pm 3.68 \times 10^1$ bacteria cm^{-2}, untreated: $9.86 \times 10^4 \pm 3.65 \times 10^4$ bacteria cm^{-2}) (one-tailed *t*-test, $P < 0.005$).

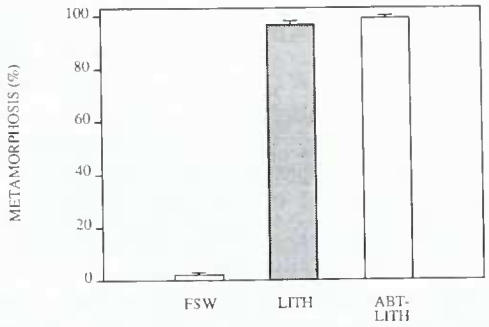

Figure 8. Mean percentage of *Strongylocentrotus droebachiensis* larvae that metamorphosed in response to filtered seawater (FSW), *Lithothamnion glaciale* (LITH), and *L. glaciale* treated with antibiotics (ABT-LITH). Each treatment consists of 5 replicates with 25 larvae per replicate. Error bars indicate standard error.

Discussion

Under static laboratory conditions, *Strongylocentrotus droebachiensis* showed a high rate of metamorphosis in response to three different morphological types of coralline red algae: a finely branched erect form (*Corallina officinalis*), a rugose crust with short nubby branches (*Lithothamnion glaciale*), and a smooth crust (*Phymatolithon*). In the field, however, passive entrapment of larvae may result in higher settlement on the more structurally complex branched and rugose corallines than on relatively smooth crusts. Dense aggregations of juveniles of *S. droebachiensis* (Scheibling, pers. obs.) and other small invertebrates (Keats *et al.*, 1984) have been observed on *C. officinalis* in the field. In eastern Newfoundland, Keats *et al.* (1984) found that juveniles of *S. droebachiensis* (2–6 mm test diameter) were most abundant on *L. glaciale* and rare on *Phymatolithon laevigatum*, *P. rugulosum*, and *Clathromorphum circumscriptum* (another smooth crust). However, the extent to which these observed distributions of juveniles in the field are determined by settlement processes or by differential mortality or migration is unknown. Flume experiments, examining settlement on algae with various morphologies, would be helpful in establishing the role of passive settlement in determining juvenile distribution patterns.

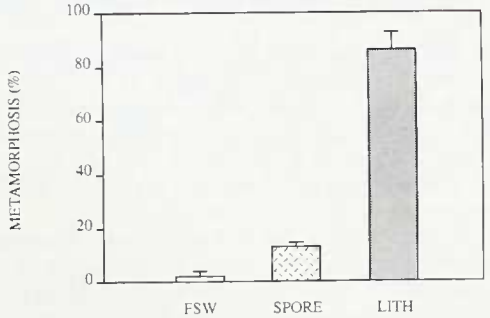

Figure 7. Mean percentage of *Strongylocentrotus droebachiensis* larvae that metamorphosed in response to filtered seawater (FSW), spores of *Lithothamnion glaciale* (SPORE), and *L. glaciale* (LITH). Each treatment consists of 5 replicates with 25 larvae per replicate. Error bars indicate standard error.

Metamorphosis of the larvae of *S. droebachiensis* appears to involve contact chemoreception. There is no evidence (from experiments with *Lithothamnion*-conditioned filtered seawater) that a chemical inducer is released into the water column (at least at concentrations that larvae can detect) or that larvae are responding to a diffusion gradient surrounding the alga. Boiling or ashing *L. glaciale* inactivates the inducing factor, suggesting that the inducer of metamorphosis of *S. droebachiensis* is a heat-labile molecule. Because these treatments kill algal tissues but do not visibly alter the surface contour of *L. glaciale* [the term contour is used to indicate that the scale of roughness is larger than the larva itself (Crisp, 1976)], thigmotaxis per se is probably not important in initiating metamorphosis.

Although induction of metamorphosis of *S. droebachiensis* may require contact with *L. glaciale*, recently metamorphosed individuals were not always located on the alga. Because larvae can be induced to metamorphose by isolated algal spores, some of these juveniles may have metamorphosed directly upon contact with spores released from *L. glaciale* onto the glass bottom of the jars. Alternatively, some larvae may land on the alga and receive a cue for metamorphosis, but then swim or crawl to adjacent areas before, or shortly after, metamorphosis. The latter phenomenon has been observed with the coral, *Agaricia tenuifolia*; the larvae require contact with the surface of crustose coralline algae to metamorphose, but subsequent attachment does not always occur directly on the algae (Morse *et al.*, 1988). In contrast, larvae of the abalone, *Haliotis rufescens*, settle and metamorphose exclusively on crustose coralline algae and not on adjacent non-algal surfaces (Morse *et al.*, 1980a).

Aqueous extracts of *L. glaciale* can induce metamorphosis of larvae of *S. droebachiensis*, indicating that

grinding releases a water-soluble chemical cue. Larvae of the sea urchin, *Strongylocentrotus purpuratus* (Rowley, 1989), are induced to settle and metamorphose in response to the same small peptide inducer, purified from extracts of crustose coralline red algae (*Lithothamnium californicum*), that induces the larvae of *H. rufescens* to metamorphose (Morse *et al.*, 1984). These surface protein-linked oligopeptides have been demonstrated to be GABA-mimetic in their interaction with the larval receptors controlling metamorphosis of *H. rufescens* (Trapido-Rosenthal and Morse, 1986). GABA also triggers the metamorphosis of *S. droebachiensis*, but at higher concentrations (10^{-4}–10^{-3} *M* range) than those recorded for *H. rufescens* (10^{-6} *M*) (Morse *et al.*, 1980b).

A metamorphosis-inducing factor may be produced by coralline algae per se or by some component of the microbial film associated with these algae. Treating *L. glaciale* with antibiotics did not reduce the rate of metamorphosis of *S. droebachiensis*, even though the number of live bacteria on the surface of the alga was reduced by two orders of magnitude. However, some residual bacteria or other microbes (such as diatoms and protozoa) unaffected by antibiotics may be responsible for the production of an inducing factor.

Other laboratory studies of *Strongylocentrotus* spp. have shown that the larvae metamorphose in response to various substrata besides coralline red algae. Larvae of *S. purpuratus* showed similar rates of metamorphosis on rocks covered with coralline red algae and those with a marine microbial film and no coralline algae (Cameron and Schroeter, 1980). Rowley (1989) found that coralline red algae and red algal turf induced similar numbers of larvae of *S. purpuratus* to metamorphose, but that metamorphosis was significantly lower with filmed rocks. We have observed a high rate of metamorphosis of larvae of *S. droebachiensis* in response to a variety of macroalgae, including non-coralline brown, green, and red algae, as well as microbial and algal films (Pearce and Scheibling, in prep.). Thus, although adults of *S. droebachiensis* are frequently associated with coralline substrata, the factors triggering metamorphosis are apparently not specific to coralline red algae. This suggests that selective settlement of *S. droebachiensis* in coralline algal barren grounds rather than kelp beds may be less important than factors that limit larval supply to kelp beds [*e.g.*, deflection of water currents by kelp plants (Jackson and Winant, 1983)], larval predation by planktivorous fish (Tegner and Dayton, 1981; Gaines and Roughgarden, 1987) and suspension feeders (Pearse *et al.*, 1970; Bernstein and Jung, 1979)], or early post-settlement survival (Cameron and Schroeter, 1980; Harris *et al.*, 1984; Rowley, 1989).

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